Original Article

# Pain Sensation in CD-1 Mice Following Long-term Consumption of Beans Diet in the Tail Flick and Hot Plate Test

**Aduema Wadioni, C F Iniodu[1], J K Opara[1]**

Departments of Human Physiology and [1]Human Anatomy, Gregory University, Uturu, Abia State, Nigeria

## Abstract

**Background and Aim:** Beans contain serotonin and its precursor 5-hydroxytryptophan (5HTP) which is known to have neurobehavioral effects on pain sensation. In the present study, we have planned to evaluate the effect of chronic consumption of beans diet on pain sensation in mice. **Methods:** Forty CD-1 mice were randomly assigned into four groups, namely, control, cooked beans diet (50% w/w), uncooked beans diet (50% w/w), while another set of mice were placed on serotonin precursor (5-HTP) diet (0.2 mg/50 g w/w) for 30 days. All the mice had access to clean drinking water *ad libitum*. Before the neurobehavioral parameters were assessed, the phytochemical analysis of the beans, $LD_{50}$ of the beans, and that of the serotonin precursor (5-HTP) were determined. Serotonin concentration was measured in the beans using gas chromatography analysis. The tail flick and hot plate test were used to assess the pain sensation. **Results:** In tail flick test, the latency of tail flick in the study group was significantly higher ($P < 0.05$) compared to the control group in both phases, thus showing a decrease in pain sensation. In hot plate test, the frequency and duration of right hind paw lick in the study group were significantly lower ($P < 0.05$) compared to control, representing a decrease in pain sensitivity. The latency of jump was significantly higher than the control, showing a decrease in pain sensitivity. **Conclusion:** Our results suggest that beans diet decreases pain sensation in mice.

**Keywords:** Beans, hot plate test, mice, pain sensation, tail flick

***Received:*** 1st February, 2017; ***Revised:*** 20th February, 2017; ***Accepted:*** 28th February, 2017

## Introduction

Pain can simply be defined as a hurtful feeling caused by damage to the tissues of the body. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[1] Pain is, therefore, a protective mechanism in the body. Beans are a good source of protein, carbohydrates, dietary fiber, minerals, vitamins, and many phenolic compounds.[2] Nowadays, researchers are particularly interested in the high antioxidant activities observed in beans. Bean is a very nutritious food from many aspects, and it is not surprising that nutritionists would characterize beans as a nearly perfect food.[3,4] It has been reported that beans have anticarcinogenic, antimutagenic,[5] anti-inflammatory, antidiabetic, hypoglycemic, depurative, cardioprotective, and antioxidant effects.[6] It has also been reported that beans contain serotonin and its precursor 5-hydroxytryptophan (5-HTP).[7]

Beans contain other chemical compounds including saponins, tannins, glycosides, and flavonoids. Among the array of chemical constituents, notably, serotonin has neurobehavioral actions such as mood, pain, and anxiety.[8] Serotonin has been shown to act (*Caenorhabditis elegans*) as a neurotransmitter to modulate behavior in response to changing cues, acting on both neurons and muscles to affect egg laying, pharyngeal pumping, locomotion, and pain.[9] Since beans contain neurotransmitters and chemicals that can potentially affect behavioral patterns, it may be worthwhile to find out whether long-term consumption of beans diet can affect the behavior in mice. This was of particular interest when we consider the challenges that confront human behavior and how behavioral disorders still remain a global concern.[10] Nigerian beans commonly called iron beans contain neurotransmitters, notably serotonin that has neurobehavioral actions as well as its precursor, 5-HTP

**Access this article online**

Quick Response Code:

Website: www.ijcep.org

DOI: 10.4103/ijcep.ijcep_8_17

**Address for correspondence:** Dr. Aduema Wadioni, Department of Human Physiology, Gregory University, Uturu, Abia State, Nigeria.
E-mail: wadioniaduema@gmail.com



**How to cite this article:** Wadioni A, Iniodu CF, Opara JK. Pain sensation in CD-1 mice following long-term consumption of beans diet in the tail flick and hot plate test. Int J Clin Exp Physiol 2017;4:21-4.

that also has similar actions. It is conceivable therefore that long-term consumption of beans diet can affect behavior such as pain sensation.

## Materials and Methods

### Experimental animals/grouping

Forty Swiss white mice weighing between 15 and 30 g were obtained from the animal house of the Department of Pharmacology, University of Nigeria, Nsukka, were used for this study. The animals were transported to the animal house of Department of Physiology, Abia State University, Uturu, Nigeria, where they were acclimatized under standard laboratory conditions and given free access to normal feed and clean drinking water. The animals were randomly assigned into four groups: Control group, Group 1, Group 2, and Group 3. The animals in the control group received normal feed, Group 1 received 50 g cooked beans per every 50 g of rodent chow, Group 2 received uncooked beans per every 50 g of rodent chow, and Group 4 received 0.2 mg/50 g serotonin precursor diet for 30 days.

### Experimental design

The tail flick and the hot plate test were used to assess the pain sensation in mice as developed by D'Amour and Smith[11] and Bertollo *et al*.[12] and were used to assess the effect of beans consumption on pain. Water was boiled to steam at 100°C, and a portion of this hot water was taken, and some cold water was added to reduce the temperature of the hot water to 49°C, in which the experiments were carried out. This temperature was constantly maintained by adding cold water when the temperature is above 49°C or by adding hot water when the temperature decreases below 49°C before each experiment was carried out. Thermometer was also constantly immersed into hot water to ensure accurate temperature is maintained. The mouse was restrained loosely, gently handled, and care was taken to prevent bite. A stopwatch was set and started exactly at the time when the tail of the mouse was immersed into hot water at 49°C. The stopwatch was stopped exactly when the mouse flicked its tails from hot water. This time was recorded as the latency of tail flick. The experiment was repeated after 1 h for each mouse. The experiments were carried out on both the control and the test groups; after the experiment, the animals were returned to their metabolic cages thereafter. The experiment lasted for a day.

Furthermore, the procedure for the hot plate test involved first turning the apparatus on and waiting a few minutes until the surface attained the required temperature, which was maintained at 55°C ± 5°C. Mice were individually exposed to the hot plate apparatus using a plastic container. The foot pedal was tapped immediately after introducing the mouse in the apparatus to start the timer and tapped again to stop the trial when the required behavior was observed. The cutoff point for the test per mouse was 30 s, in case the required behavior was not observed. This was to avoid extensive tissue damage.[12] The behavior measured was the time it took for the mice to start licking their footpad. This behavior, defined as the latency of hind paw lick, was recorded. The behavior observed, paw lick and jumping are the most common measures of pain threshold and are considered supraspinally integrated.[13-15]

### Statistical analysis

Data collected during the study were expressed as a mean ± standard error of the mean. Analysis of variance and Student's *t*-test were used for analysis, and $P < 0.05$ was considered statistically significant. Furthermore, *post hoc* test (<standard deviation) was carried out. Statistical analysis was done with the aid of computer software SPSS (Brain Series, China) and Excel from Windows XP.

## Results

Figure 1 shows the latency of tail flick (the time taken by the mice to flick its tail from the warm water) by mice fed with normal diet, cooked beans, uncooked beans, and serotonin precursor diet. The values were 10.32 ± 3.66, 15.45 ± 2.86, 18.56 ± 2.26, and 18.99 ± 3.16 s in Trial 1 and 8.30 ± 2.07, 12.40 ± 2.82, 21.20 ± 3.90, and 24.31 ± 1.96 s for mice fed with normal diet, cooked beans, uncooked beans, and serotonin precursor diet in Trial 2, respectively. The latency of tail flicks in Trial 1 was significantly higher in Groups 1, 2, and 3 ($P < 0.05$) when compared with that of the control group. However, the latency of tail flicks in serotonin precursor fed (Group 3) mice was significantly higher when compared to that of cooked beans group of mice. In Trial 2, the latency of tail flicks was significantly higher ($P < 0.05$) in Groups 1, 2, and 3 when compared with that of the control group. Among Groups 1, 2, and 3, the latency of mice fed with uncooked beans and serotonin precursor group was significantly higher compared to that of cooked beans group of mice.

Figure 2 shows the frequency of hind paw licks in the control group and Groups 1, 2, and 3. The number of times the animals (mice) in both groups' licks their hind paw in the hot plate test was 4.00 ± 0.52, 3.25 ± 0.65, 2.38 ± 0.63, and 1.29 ± 0.29 s

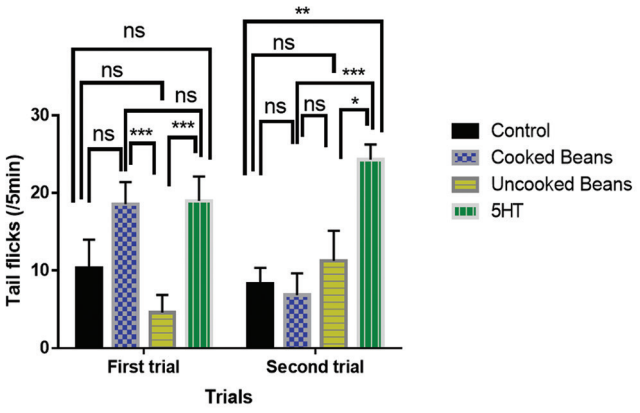


**Figure 1:** Latency of tail flick among the different experimental groups for assessing pain during the tail flick test. Data were statistically analyzed using one-way analysis of variance with Tukey's multiple comparison test (*post hoc*)

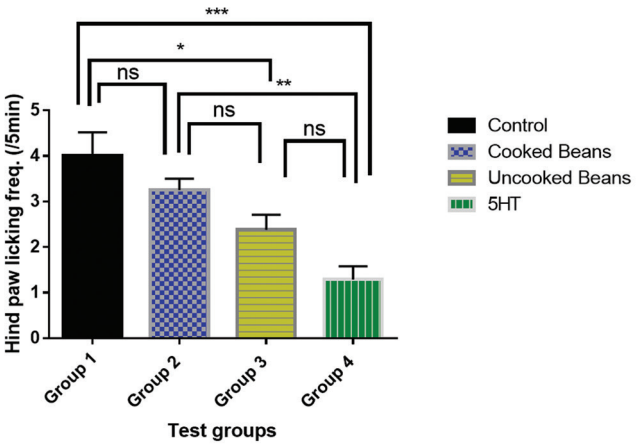


**Figure 2:** Frequency of hind paw lick among the different experimental groups for assessing pain during the hot plate test. Data were statistically analyzed using one-way analysis of variance with Tukey's multiple comparison test (*post hoc*)

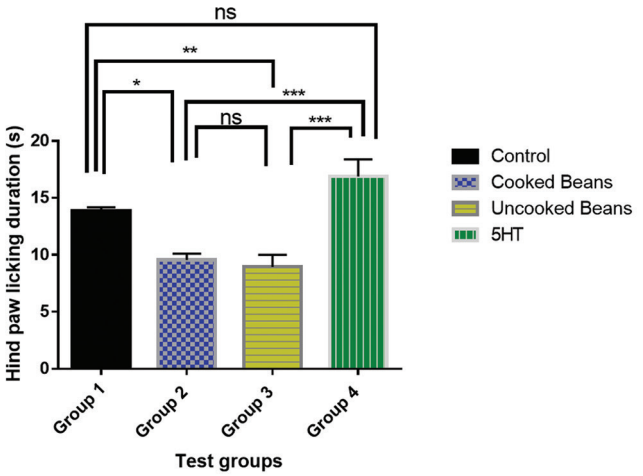


**Figure 3:** Duration of hind paw lick among the different experimental groups for assessing pain during the hot plate test. Data were statistically analyzed using one-way analysis of variance with Tukey's multiple comparison test (*post hoc*)

for mice fed with normal diet, cooked beans, uncooked beans, and serotonin precursor diet, respectively. The frequency of hind paw licks for mice fed with beans and serotonin precursor diet was significantly less when compared with that of the controls ($P < 0.05$). However, the serotonin precursor group had a significantly less frequency of hind paw licks when compared with that of the cooked beans group of mice.

Figure 3 shows the duration of hind paw licks. The duration taken by the animals (mice) in the control group and Groups 1, 2, and 3 to lick their hind paw in the hot plate test was 16.89 ± 1.50, 13.84 ± 02.34, 9.56 ± 1.24, and 8.95 ± 2.05 s, respectively. The mice fed with cooked, uncooked beans, and serotonin precursor fed mice had a significantly lesser ($P < 0.05$) duration of hind paw licks when compared with that of controls. However, the mice fed with uncooked beans and serotonin precursor group had a significantly lesser duration of hind paw licks compared to that of cooked beans group of mice.

The latency of jump in the hot plate test for mice fed with normal, cooked, uncooked beans, and serotonin precursor diet were 14.80 ± 3.04, 18.76 ± 3.92, 20.21 ± 4.22, and 25.70 ± 1.40 s. The latency of jump by the cooked beans, uncooked beans, and serotonin precursor fed mice was significantly higher ($P < 0.05$) when compared to that of the control group. Among the mice fed with cooked beans, uncooked beans, and serotonin precursor, the serotonin precursor fed mice had a significantly higher latency of jump in the hot plate test when compared to that of cooked and uncooked beans group of mice [Figure 4].

## Discussion

In the present study, the animal models of physiological pain assessment used include tail flick test and hot plate test.[12] Tail flick is a system feature that involves when radiant heat is applied on the animal's tail - when the animal feels discomfort, there is a sudden tail movement (tail flick). This has proved that it is particularly a sensational property of pharmacological substance. It can also be used to evaluate basal thermal pain sensitivity or the study of putative genetic differences among animals without drugs.[16] In the experiment, the tail flick test showed that the latency of tail flick for the group of mice fed with cooked beans diet was significantly higher than the control. This indicates that the pain threshold for the test mice was raised when compared to the control, implying that mice took a longer time before they perceived pain. These longer latencies indicate a raised pain threshold and thus decreased pain perception following the consumption of uncooked beans diet. This shows that beans affect the spontaneous response to the sensation of pain.

The hot plate paw-shaking and paw-licking is a complex and supraspinal organized behavior. The latency of jump in the hot plate (heated to and maintained at 55°C to prevent excessive tissue damage) was defined as the time taken by the mice to jump after being introduced into the hot plate. The hot plate procedure is believed to have an advantage over other thermal nociceptive pain model such as tail flick procedure[11] because it can be applied repeatedly in the same animal over a short period of time (2–3 h) without causing tissue damage or injury, especially if the maximum observation time is 30 s. It also constitutes a more global estimate of nociceptive reactivity because it represents a complex pattern of willed behavior rather than a simple reflex, like the tail flick.[17]

In the hot plate test, the latencies of jump by the cooked beans, uncooked beans, and serotonin precursor diet groups of mice were significantly longer than that of the control mice that consumed normal rodent chow, indicating that it took longer time for beans fed mice to perceive pain than the control group of mice. This shows that beans had an analgesic effect on mice. The serotonin circuitry is a well-established pathway involved in brain's analgesia system during transmission of pain in the

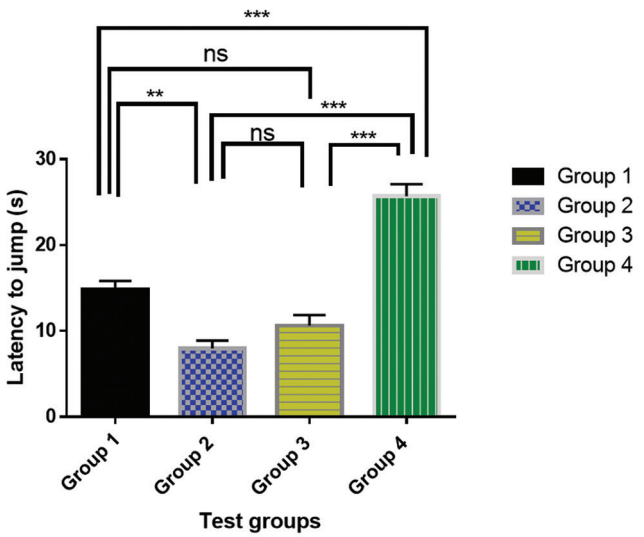


**Figure 4:** Latency of jump among the different experimental groups for assessing pain during the hot plate test. Data were statistically analyzed using one-way analysis of variance with Tukey's multiple comparison test (*post hoc*)

central nervous system. It is known that the analgesic fibers of these system release neurotransmitters that inhibit pain transmission to the brain, and the neurotransmitters released by the fibers of analgesic pathway are serotonin and enkephalins.[1,18] This result suggests that the mice fed with beans diet were less sensitive to pain when compared to those fed with the control diet. The decrease in the sensitivity to pain caused by the beans diet may also be attributed to the presence of flavonoids and phlobatannins in the beans which has been reported to reduce pain perception due to their anti-inflammatory properties.[19,20]

### Limitations of the study

My inability to use humans for this experiment is a major limitation as the results in mice will only be extrapolated to humans, because of their natural (mammalian) similarity to humans.

## Conclusion

Chronic consumption of beans diet decreases pain sensation. If this result is extrapolated to humans, long-term consumption of beans diet may be beneficial in the control and management of pain in humans. Further research is, however, recommended to confirm if beans (*Vigna unguiculata*) can be used as a pain killer. Our findings suggest that beans diet reduces pain sensation in mice.

### Acknowledgment

We acknowledged Pa and Mrs. B. A. Aduema, Mr. Iwasam Joshua, Dr. Nmaju, Prof. E. E. Osim, and Associate Prof. A. A. Nwankwo, for their priceless support.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Osim EE. Neurophysiology. Calabar: University of Calabar Press; 2008. p. 24-7.
2. Adeyele EJ. Studies of Chemical Composition and Functional Properties of African Yambeans (Spensotylis Stenoorpa) Flour. PhD Thesis, Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria; 1995.
3. Shamsuddin AM, Elsayed AM, Ullah A. Suppression of large intestinal cancer in F344 rats by inositol hexaphosphate. Carcinogenesis 1988;9:577-80.
4. Vander Poel AF, Mollee PW, Huisman J, Liner IE. Variations among species of animals in response to the feeding of heat-processed beans. Bean processing and effects on growth, digestibility and organ weights in piglets. Livest Prod Sci 1990;25:121-35.
5. Graf E, Eaton JW. Suppression of colonic cancer by dietary phytic acid. Nutr Cancer 1993;19:11-9.
6. Bennick E, Maurice O, Elizabeth R. Beans & Health: A Comprehensive Review. Frazee, MN: Churchill, Livingstone; 2008.
7. Portas CM, Bjorvatn B, Ursin R. Serotonin and the sleep/wake cycle: Special emphasis on microdialysis studies. Prog Neurobiol 2000;60:13-35.
8. Brunton LB, Lazio JS, Parker KL. Therapeutics. The Pharmacological Basis of Therapeutics. New York: McGraw-Hill; 2005. p. 607-29.
9. Chase DL, Koelle MR. Biogenic amine neurotransmitters in *C. elegans*. WormBook 2007;3:1-15.
10. Messman T. Psychiatric Drugs: Chemical Warfare on Humans: Interview with Robert Whitaker. Available from: http://www.naturalnew.com. [Last retrieved on 2005 Aug 27].
11. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74-9.
12. Bertollo CM, Oliveira AC, Rocha LT, Costa KA, Nascimento EB Jr., Coelho MM. Characterization of the antinociceptive and anti-inflammatory activities of riboflavin in different experimental models. Eur J Pharmacol 2006;547:184-91.
13. Elmer GI, Pieper JO, Negus SS, Woods JH. Genetic variance in nociception and its relationship to the potency of morphine-induced analgesia in thermal and chemical tests. Pain 1998;75:129-40.
14. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev 2001;53:597-652.
15. Lima LM, Perazzo FF, Tavares Carvalho JC, Bastos JK. Anti-inflammatory and analgesic activities of the ethanolic extracts from *Zanthoxylum riedelianum* (*Rutaceae*) leaves and stem bark. J Pharm Pharmacol 2007;59:1151-8.
16. Adrianbeloson R. Evaluation pain sensitivity amonganimals. Neuropsychopharmacol Psychiatry 2006;24:459-560.
17. Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, *et al.* Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci 1999;19:3248-57.
18. Sembulingam K, Sembulingam P. Neurophysiology of pain. Essentials of Physiology. New Delhi: Jaypee Brothers, Medical Publishers; 2010. p. 803-10.
19. Xu HX, Lee SF. Activity of plant flavonoids against antibiotic-resistant *Bacteria*. Phytother Res 2001;15:39-43.
20. Muller H. Hindu Medicine. Baltimore: The John Hopkins Press; 1992.